PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ULB-009-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/00493	International filing date *(day/month/year)* 20.01.2003	Priority date *(day/month/year)* 18.01.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/10		
Applicant UNIVERSITE LIBRE DE BRUXELLES et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 9 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 5 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
13.08.2003	08.07.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Gabriels, J Telephone No. +31 70 340-4282

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/00493

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-47 as originally filed

Claims, Numbers

1-28 received on 01.06.2004 with letter of 21.05.2004

Drawings, Sheets

1/27-27/27 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-28
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-28
Industrial applicability (IA)	Yes:	Claims	1-28
	No:	Claims	

2. Citations and explanations

   see separate sheet

I. Basis (Continuation)

The Sequence listing pages 1-14 filed with the letter of 04/04/2003 do not form part of the application (Rule 13ter.1(f) PCT).

IV. Lack of unity (Continuation)

3.1 This International Preliminary Examining Authority agrees with the findings of the International Searching Authority as to the lack of unity. The multiple (groups of) inventions found in this international application, are as follows: **Invention 1:** claims 1-12 Methods and kits for isolating quantifiable in vivo RNA from a biological sample. **Invention 2:** claims 13-28 Methods and kits for the diagnosis/ prognosis/ monitoring of a clinical status affecting the immune system, for the screening for compounds modulating the clinical status affecting the immune system, and uses thereof.

3.2 The common "essential technical feature" shared by all the claims is the use of an automated setup for improving methods for isolating quantifiable in vivo nucleic acids from a biological sample. Automation of these methods in order to improve these methods is obvious to the person skilled in the art (see section V 2.3.3.1).

3.3 Therefore, two problems can be defined in the present application : **1)** A first problem is the isolation of quantifiable in vivo RNA from a biological sample. **2)** A second problem is the diagnosis/ prognosis/ monitoring of a clinical status affecting the immune system and the screening for compounds modulating this clinical status.

3.4 In view of the fact that methods for isolating quantifiable in vivo nucleic acids from a biological sample are already well known in the prior art, due to the intrinsic differences between the different problems described above, and due to the fact that no other technical features can be distinguished which, in the light of the prior art, could be regarded as "special technical features" common to the solutions of these different problems, the IPEA is of the opinion that there is no single inventive concept underlying the 3 inventions of the present application in the sense of rule 13.1 PCT.

3.5 The applicant payed an additional search and examination fee for invention 2.

Therefore inventions 1 and 2 listed above (claims 1-28) have been searched and examined.

V. Reasoned statement (Continuation)

2.1 CITATIONS

Reference is made to the following documents:

D1: WO 02 00599 A (QIAGEN GMBH ;HOLLAENDER VERA (DE); OELMUELLER UWE (DE); WYRICH RAL) 3 January 2002 (2002-01-03)

D2: WO 94 18156 A (UNIV IOWA RES FOUND) 18 August 1994 (1994-08-18)

D3: US-A-5 906 744 (CARROLL RICHARD J ET AL) 25 May 1999 (1999-05-25) cited in the application

D4: DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 16 November 2001 (2001-11-16) RAINEN LYNNE ET AL: 'Stabilization of expression profiles of genes associated with the inflammatory response in post-phlebotomy whole blood using the PAXgeneTM blood RNA system' Database accession no. PREV200200151792 XP002260618 & BLOOD, vol. 98, no. 11 Part 2, 16 November 2001 (2001-11-16), pages 108b-109b, 43rd Annual Meeting of the American Society of Hematology, Part 2;Orlando, Florida, USA; December 07-11, 2001 ISSN: 0006-4971

D5: WO 99 49083 A (UROCOR INC) 30 September 1999 (1999-09-30)

D6: WO 99 57130 A (GENE LOGIC INC ;PRASHAR YATINDRA (US); WEISSMAN SHERMAN (US)) 11 November 1999 (1999-11-11)

D7: WO 94 28123 A (ONTARIO CANCER INST ;THOMPSON CRAIG BERNIE (US)) 8 December 1994 (1994-12-08)

2.2 NOVELTY (Art. 33(2) PCT)

2.2.1 **Invention 1:** claims 1-12 Methods and kits for isolating quantifiable in vivo RNA from a biological sample.

2.2.2 D1 discloses novel compositions for isolating and/or stabilising nucleic acids in

material of a biological origin. The investigation of RNA expression patterns in microorganisms by molecular-biological methods such as e.g. quantitative RT-PCR, NASBA, bDNA technology or biochips and Northern Blotting is used in basic research in the analysis of gene expression in prokaryotes, as well as in protozoa, fungi and algae and has also acquired increasing importance for example in medical diagnosis. There is the problem that in order to isolate the nucleic acids the organisms have to be removed from their natural environment in order to obtain the cells for investigation and these then have to be transported to the place for isolation of the nucleic acid. At the same time there is a major risk that the RNA profiles and also the DNA will change. This would lead to wrong diagnosis or analysis of, for example, gene expressions in bacterial cultures or, for example, in medical/clinical diagnosis in an investigation of infected patient material (e.g. samples taken from sites of inflammation) forming the basis for the analysis of nucleic acids, or foods contaminated with bacteria, fungi, protozoa or algae. In food samples or clinical samples from patients the microorganisms may even die and the nucleic acids, particularly the RNA, are then broken down entirely. Therefore it is of maximum importance for the nucleic acids, particularly the RNA, to be stabilised immediately after the sample is taken. In the examples, D1 discloses the use of tetradecyltrimethylammonium oxalate, RNeasy Mini spin columns, and commercial buffers based on a guanidinium salt such as e.g. guanidinium isothiocyanate. However, D1 does not use an automated setup or device.

2.2.3 D2 discloses a method for the quantification of in vivo RNA from a biological sample. As an example D2 discloses the isolation of oncogene RNA. Chronic myelogenous leukemia cells express an oncogene (bcr/abl) which is expressed in the immortal leukemic cell line K562 but not in normal cells. To demonstrate the utility of the invention in the isolation of RNA for the detection of RNA species by reverse transcriptase and polymerase chain reaction (PCR), 30-10,000 K562 cells were mixed with 200 microliters whole blood. These blood samples were: a) collected in a tube comprising a compound inhibiting RNA degradation and/or gene induction, (b) forming a precipitate comprising nucleic acids, (c) separating said precipitate of step (b) from the supernatant, (d) dissolving said precipitate of step (c) using a buffer, forming a suspension, (e) isolating nucleic acids from said suspension by centrifugation. The isolated RNA was resuspended, and used for subsequent RT-PCR (cf. examples 1, 5, and 11). Furthermore, D2 discloses the use of tetradecyltrimethylammonium oxalate as quaternary ammine surfactant (cf. example 1) and the use of guanidinium isothiocyante containing buffers (cf. claim

12). However, D2 does not use an automated setup or device.

2.2.4 The present application meets the criteria of Article 33(1) PCT, because the subject-matter of claims 1-12 is new in the sense of Article 33(2) PCT.

2.2.5 **Invention 2:** claims 13-28 Methods and kits for the diagnosis/ prognosis/ monitoring of a clinical status affecting the immune system, for the screening for compounds modulating the clinical status affecting the immune system, and uses thereof.

2.2.6 D4 discloses that a major impediment to accurate analysis of gene expression in whole blood is the post-phlebotomy change in cellular transcript patterns. Nuclease degradation of RNA as well as non-specific induction of is triggered minutes after blood collection and continues during sample transport and processing. D4 discloses an evacuated blood collection tube containing a stabilizing additive and a chemically linked companion sample processing system, the PAXgeneTM Blood RNA System (PAXgene) that: 1) stabilizes cellular RNA in whole blood and, 2) purifies, via spin-column technology, high quality total RNA. D4 compares gene expression profiles of room temperature stored PAXgene and unpreserved (EDTA) whole blood using quantitative PCR (ABI Prism 7700 TaqManTM) assays for 36 gene transcripts associated with inflammation. RNA was purified from PAXgene and unpreserved EDTA whole blood at the time of phlebotomy (to) and 4, 8, 24, 72 and 120 hours post-phlebotomy. Eleven of 36 transcripts were undetectable in either PAXgene or EDTA blood. mRNA expression levels were unchanged in 3 of 25 measurable transcripts in both donors in EDTA blood; the relative amount of each of the remaining 22 transcripts changed by up to 104-fold. By contrast, 23 of 25 measurable transcripts were stable in PAXgene blood in one or both donors for 5 days at ambient temperature. The %CV of the PAXgene Blood RNA System for three donors (8 tubes/donor) were 7-24% for total RNA yield and 1% for Ct values in a GAPDH RT PCR assay. The ability to stabilize and purify high quality RNA using the PAXgene Blood RNA System, coupled with Source Precision Medicine's optimized quantitative PCR, enables routine use of gene expression analysis in the evaluation of clinical samples.

2.2.7 D5 discloses diagnostic techniques for the detection of human disease states that affect gene expression in peripheral leukocytes. The invention relates particularly to probes and methods for evaluating the presence of RNA species that are

differentially expressed in the peripheral blood of individuals with such a disease state compared to normal healthy individuals. An example of such a marker is a product of an interleukin 8 (IL-8) or interleukin 10 (IL-10) gene (cf. claim 18).

2.2.8 D6 discloses a method to identify a therapeutic or prophylactic agent that modulates the response of a lymphocyte population to an antigen (cf. claim 1).

2.2.9 D7 discloses a method of screening a drug for immunomodulatory effects using the expression level of the gene encoding CD28 (cf. claim 7).

2.2.10 The present application meets the criteria of Article 33(1) PCT, because the subject-matter of claims 13-28 is new in the sense of Article 33(2) PCT.

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 The document D4 is regarded as being the closest prior art to the subject-matter of claims 1, 10, 13, 15, 16, 19, 20, 22, and 24. The subject-matter of these claims differs from D4 in that the extraction and detection of the nucleic acids is performed using an automated setup.

2.3.2 The problem to be solved by the present invention may therefore be regarded as providing a more reliable method for in vivo quantification of RNA from a biological sample. The solution would be the automation of the extraction and detection process.

2.3.3 The solution proposed in claims 1, 10, 13, 15, 16, 19, 20, 22, and 24 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons.

2.3.3.1 The present application merely combines the advantageous of two well known methods (i.e. the PAXgeneTM Blood RNA System and the MagNA Pure LC mRNA Isolation Kit I). The statement In the PAXgene protocol concerning the recommended use of the PAXgene Blood RNA Tube with the PAXgene Blood RNA kit merely reflects the procedure that is guaranteed by the supplier of the kit. The person skilled in the art knows the problems associated with methods for determining in vivo RNA levels in a biological sample. These problems include the stabilization and fixation of the RNA sample. In addition, the skilled person knows that the automation and simplification of the process contribute to an improved

quantification of the RNA sample. These problems have been solved by the PAXgeneTM Blood RNA System for better stabilization of the sample and by the MagNA Pure LC mRNA Isolation Kit I in combination with the MagNA pure LC Instrument for the automated process. It would be obvious for the skilled person that the combination of both methods would further enhance the detection of in vivo RNA levels in a biological sample. Furthermore, the combination of both methods starting from the precipitate is the most logical and therefore obvious solution for combining both methods.

2.3.4 Dependent claims 2-9, 11, 12, 14, 17, 18, 21, 23, 25-27, and 28 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of novelty and/or inventive step.

2.3.5 The present application **(invention 1)** does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1-12 does not involve an inventive step (Rule 65(1)(2) PCT).

2.3.6 The present application **(invention 2)** does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 13-27, and 28 does not involve an inventive step (Rule 65(1)(2) PCT).

We Claim (retyped)

1. A method for the quantification of *in vivo* RNA from a biological sample comprising the steps of:

(a) collecting said biological sample in a tube comprising a compound inhibiting RNA degradation and/or gene induction,

(b) forming a precipitate comprising nucleic acids,

(c) separating said precipitate of step (b) from the supernatant,

(d) dissolving said precipitate of step (c) using a buffer, forming a suspension,

(e) isolating nucleic acids from said suspension of step (d) using an automated device,

(f) dispersing/distributing a reagent mix for RT-PCR using an automated device,

(g) dispersing/distributing the nucleic acids isolated in step (e) within the dispersed reagent mix of step (f) using an automated device, and,

(h) determining the *in vivo* levels of transcripts using the nucleic acid/RT-PCR reagent mix of step (g) in an automated setup.

2. The method according to claim 1, wherein steps (a) and (b) are performed simultaneously.

3. The method according to claim 1 or 2, wherein the compound of step (a) comprises a quaternary amine surfactant.

4. The method according to claim 3, wherein said quaternary amine is tetradecyltrimethyl-ammonium oxalate.

5. The method according to any of the claims 1 to 4, wherein the tube of step (a) is an open or a closed blood collecting tube.

6. The method according to any of the claims 1 to 5, wherein the buffer of step (d) is a guanidine-thiocyanate-containing buffer.

7. The method according to any of the claims 1 to 6, wherein the isolation of nucleic acids of step (e) is performed using RNA-capturing beads.

8. The method according to any of the claims 1 to 7, wherein said *in vivo* levels are determined using real time PCR.

9. The method according to any of the claims 1 to 8, wherein said quantification is performed using a biological sample of 100 µl.

10. A kit for isolating quantifiable *in vivo* RNA from a biological sample comprising:
(a) optionally, a collection tube for biological samples,
(b) a compound inhibiting RNA degradation and/or gene induction,
(c) reagents for automated RNA isolation,
(d) a reagent mix for a simultaneous RT and real-time PCR reaction or separate compounds thereof , allowing the automated dispersion of said mix,
(e) optionally, specific oligonucleotides to perform said RT-PCT reactions, and,
(f) optionally, an instruction manual describing a method for an automated RNA isolation, a method for the automated dispersion of a reagent mix and the dispersion of the isolated nucleic acids for RT- real time PCR, and a method for automated RNA analysis.

11. The kit according to claim 10, wherein the compound of part (b) is a compound as defined in the methods of claims 3 or 4.

12. The kit according to claims 10 and 11, wherein additionally a buffer is provided as defined in the method of claim 6.

13. Use of a method according to any of the claims 1 to 9 or a kit according to any of the claims 10 to 12 for the monitoring/detection of changes of *in vivo* nucleic acids levels in a biological agent present in a biological sample.

14. The use of a method or a kit according to claim 13 wherein said biological agent is chosen from a group consisting of eukaryotic cells, prokaryotic cells, viruses and phages.

15. Use of a method according to any of the claims 1 to 9 or a kit according to any of the claims 10 to 12 for the monitoring/detection of changes of *in vivo* nucleic acids of a biological agent in a biological sample, in order to diagnose a certain disease.

16. Use of a method according to any of the claims 1 to 9 or a kit according to any of the claims 10 to 12 for the monitoring/detection of changes of *in vivo* nucleic acids of a biological agent in a biological sample, in order to screen for a compound for the production of a medicament for curing a disease.

17. The use of a method or a kit according to claim 15 and/or 16, wherein said disease is an immuno-related disease.

18. Use of a method or a kit according to claim 16 ~~23~~, for the detection/monitoring/screening of a compound, wherein said compound is an immunomodulatory compound which may be chosen from a group consisting of eukaryotic cells, prokaryotic cells, viruses, phages, parasites, drugs (natural extracts, organic molecule, peptide, proteins, nucleic acids), medical treatment, vaccine and transplants.

19. Use of a method according to any of the claims 1 to 9 or a kit according to any of the claims 10 to 12, for the detection/monitoring of epitope specific CTLs or immuno-related transcripts.

20. A method to identify an agent capable of modifying the immunological status of a subject:

(a) collecting *in vitro* pulsed blood cells present in a whole blood sample obtained from a subject wherein a first immunomodulatory agent(s) has been applied with a second identical/similar and/or different immunomodulatory agent or collecting *in vitro* non-pulsed blood cells present in a whole blood sample from a subject wherein a first immunomodulatory agent(s) has been applied in a tube comprising a compound inhibiting RNA degradation and/or gene induction, or adding said compound to the pulsed/ non-pulsed cells,

(b) forming a precipitate comprising nucleic acids,

(c) separating the precipitate of step (b) from the supernatant,

(d) dissolving the precipitate of step (c) using a buffer, forming a suspension,

(e) isolating nucleic acids from thesuspension of step (d) using an automated device,

(f) dispersing/distributing a reagent mix for RT-PCR using an automated device,

(g) dispersing/distributing the nucleic acids isolated in step (e) within the dispersed reagent mix of step (f) using an automated device,

(h) detecting/ monitoring/ analyzing the *in vivo* levels of immuno-related transcripts in the dispersed solution of step (g) in an automated setup, and,

(i) identify agents able to modify the immunological status of said subject,

wherein, in case the first agent of step (a) is already present in the subject, the application of said agent in said subject is omitted.

21. The method according to claim 20, wherein the agent capable of modifying the immunological status of a subject is identified via the analysis of epitope specific CTLs, and, wherein the immuno-related transcript of step (h) is an epitope specific CTL-related transcript.

22. An *in vitro* method for the diagnosis/ prognosis/ monitoring of a clinical status affecting the immune system in a subject comprising the steps of :

(a) providing sampled whole blood from said subject in a tube comprising a compound inhibiting RNA degradation and/or gene induction, or adding said compound to the blood cells,

(b) forming a precipitate comprising nucleic acids,

(c) separating the precipitate of step (b) from the supernatant,

(d) dissolving the precipitate of step (c) using a buffer, forming a suspension,

(e) isolating nucleic acids from the suspension of step (d) using an automated device,

(f) dispersing/distributing a reagent mix for RT-PCR using an automated device,

(g) dispersing/distributing the nucleic acids isolated in step (e) within the dispersed reagent mix of step (f) using an automated device,

(h) detecting/ monitoring/ analyzing the *in vivo* levels of immuno-related transcripts in the dispersed solution of step (g) in an automated setup, and,

(i) detecting/ monitoring the change in *in vivo* levels of immuno-related transcripts, and,

(j) diagnosing/ prognosing/ monitoring the disease affecting the immune system.

23. The method according to claim 22, wherein step (a) comprises the steps of :

(a) pulsing blood cells present in a whole blood sample taken from a subject with an identical/ similar and/or different immunomodulatory agent as present in the subject, and,

(b) collecting pulsed blood cells of step in a tube comprising a compound inhibiting RNA degradation and/or gene induction, or adding said compound to the pulsed cells.

24. The use or the method according to any of claims 17 to 23, wherein the immuno-related disease is chosen from the group consisting of autoimmunity, rheumatoid arthritis, multiple sclerosis, cancer (eg. in cancer immunotherapy), immunodeficiencies (eg. in AIDS), allergy, graft rejection or Graft versus Host Disease (GVHD) (eg. in transplantation), or wherein the immunomodulatory compound or agent influences one of said diseases; or wherein the change of the immuno-related transcripts or the epitope specific CTLs-related or T Helper lymphocyte-related transcripts indicate the presence of one of said diseases; or wherein the immunological status illustrates the status of one of said diseases .

25. The use or the method according to claim 24, wherein said immuno-related transcript is chosen from the group consisting of nucleic acids coding for chemokine, cytokine, growth factors, cytotoxic markers, transcription factors, members of the TNF-related cytokine-receptor superfamily and their ligands, apoptosis markers; immunoglobulins, T-cell receptor, and any marker related to the activation or the inhibition of the immune system known or to be discovered.

26. The use or the method according to claim 25, wherein said nucleic acid codes for a marker chosen from the group consisting of IL-1ra, IL-1β, IL-2, IL-4, IL-5, IL-9, IL-10, IL-12p35, IL-12p40, IL-13, TNF-α, IFN-γ, IFN-α, TGF-β, and any interleukin or cytokine involved or not in the immune response.

27. The use or the method according to claim 24, wherein said epitope specific CTLs-related or T Helper lymphocyte-related transcript is chosen from the group consisting of nucleic acids coding for cytokines, cytokine receptors, cytotoxines, inflammatory or anti-inflammatory mediators, members of the TNF-related cytokine-receptor superfamily and their ligands, G-protein coupled receptors and their ligands, tyrosine kinase receptors and their ligands, transcription factors, and proteins involved in intra-cellular signaling pathways.

28. The use or the method according to claim 27, wherein said nucleic acid codes for a marker chosen from the group consisting of granzyme, perforines, prostaglandins, leukotrienes, immunoglobulin and immunoglobulin superfamily receptors, Fas and Fas-ligand, T cell receptor, chemokine and chemokine receptors, protein-tyrosine kinase C, protein-tyrosine kinase A, Signal Transducer and Activator of Transcription (STAT), NF-kB, T-bet, GATA-3, Oct-2.